Hemolysis is the disruption of the blood cell membranes, with the release of the cell contents and membrane fragments into the surrounding fluid. Unfortunately, in most clinical laboratories, hemolysis interferes with a variety of measurements made on analytes occurring in patient specimens, making it difficult to interpret results correctly. Clinical laboratory personnel modify patient results with quantitative estimates of hemolysis as an aid to physicians in interpreting patient results, but in some situations, such as whole blood measurements, the occurrence of hemolysis is not immediately obvious and cannot be estimated.

With increased emphasis on laboratory testing, clinical laboratory procedures must be either less susceptible to interferences or be accurate and consistent to identify and deal with interfering substances so that clinicians can provide appropriate treatments without concern for patient safety. Because hemolysis is a major test interferent and the most frequent cause of specimen rejection, accurate laboratory results require that specimens be free of hemolysis or account for the effects of hemolysis.

Even though laboratorians have tried to improve the quality of their procedures, in many institutions, blood collection decentralization from laboratory personnel to nonlaboratory personnel has resulted in increasing rates of hemolysis. In addition, laboratorians have not taken full advantage of automation to estimate accurately the degree of hemolysis nor taken appropriate, consistent actions when communicating the presence of hemolysis to clinicians.

Appropriate, consistent communication in this setting can have often-overlooked patient safety consequences. Not all hemolyzed specimens can be attributed to poor specimen-collection techniques because more than 50 medical conditions can cause in vivo hemolysis. In vivo hemolysis can be masked by the occurrence of hemolysis from the use of improper collecting and processing methods for specimens. In these circumstances, specimens hemolyzed because of a medical condition may be rejected because hemolysis had been incorrectly ascribed to improper specimen phlebotomy, without considering whether the patient could be critically ill from a disease or condition causing in vivo hemolysis.
In this study, we sought to quantify the frequency of hemolyzed specimens, to identify the methods for detecting the presence and amount of hemolysis, to characterize the current responses of laboratory personnel to the appearance of hemolysis, and to suggest practice improvements.

**MATERIALS AND METHODS**

Members of the College of American Pathologists (CAP) Quality Practices Committee developed an online questionnaire, in the usual CAP Q-Probes format, which was sent to participants in the 2011 CAP C1, C3, C3X, CZ, CZX, CZ2, and CZ2X chemistry surveys. One reminder notice was sent to nonrespondents encouraging participants to complete their questionnaires and return them to the Q-Probes CAP committee staff, who then tabulated the results according to previously established Q-Probes procedures. The questionnaire contained 28 multiple-choice questions, with “other, please list” as a choice in 7 questions. If participants did not answer specific questions, they were excluded from the data only for the question(s) they did not answer.

**RESULTS**

Of the 3495 CAP customers enrolled in the Chemistry Surveys, 846 (24%) responded. Seen in Figure 1 are hemolysis rates for 772 laboratories. In 276 laboratories (36%), the overall hemolysis rate was less than 1%; in 267 laboratories (35%), it was 1% to 2.9%; and in 8 laboratories (1%), it was 15.0% or greater. In 71 laboratories (9%), personnel were unsure of the overall hemolysis rate.

A visual scale of evaluating hemolysis was used in 369 of 779 (48%) of the responding clinical laboratories, an instrument scale was used in 87 laboratories (11%), and both visual and an instrument scales were used by 323 (41%) of responders. As shown in the Table, when visually grading a specimen for hemolysis, a picture of the degree of hemolysis was used to classify each specimen in 271 of 680 laboratories (40%); however, the visual grading of specimens for hemolysis was included as part of the clinical laboratories' competency assessment program in 120 of 678 laboratories (18%). In 64 of 756 laboratories (8%), a manual procedure, such as placing a drop of specimen on gauze, followed by visual inspection, was used to differentiate hemolysis from icterus in difficult cases. Of 748 participants, 269 (36%) forwarded automated hemolysis flags that accompany hemolyzed results from their major chemistry instrument to the patients' medical record. For all analytes measured on their primary chemistry analyzers in 777 laboratories, 632 laboratories (81%) used the same hemolysis scales, 495 of 672 (74%) used the same hemolysis scales.

**Procedures Used in Identification of Hemolysis**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Total, No.</th>
<th>Answered Yes, No. (%)</th>
<th>Answered No, No. (%)</th>
<th>Answered Unsure, No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>When visually grading a specimen for hemolysis, is a picture of the degree of hemolysis used to classify each specimen?</td>
<td>680</td>
<td>271 (40)</td>
<td>389 (57)</td>
<td>20 (3)</td>
</tr>
<tr>
<td>Is the visual grading of specimens for hemolysis included as part of your laboratory's competency assessment program?</td>
<td>678</td>
<td>120 (18)</td>
<td>494 (73)</td>
<td>64 (9)</td>
</tr>
<tr>
<td>Are any manual procedures used to identify the presence of hemolysis in difficult cases (ie, place a drop of specimen on gauze, inspect the color and differentiate between hemolysis and icterus [bilirubin]?)?</td>
<td>756</td>
<td>64 (8)</td>
<td>658 (87)</td>
<td>34 (4)</td>
</tr>
<tr>
<td>Is your laboratory able to forward automated hemolysis flags that accompany hemolyzed results recorded on your major chemistry instrument to the patients' medical records?</td>
<td>748</td>
<td>269 (36)</td>
<td>378 (50)</td>
<td>101 (14)</td>
</tr>
<tr>
<td>Does your laboratory use automated result verification for the primary chemistry analyzer?</td>
<td>830</td>
<td>266 (32)</td>
<td>547 (66)</td>
<td>17 (2)</td>
</tr>
<tr>
<td>Does your laboratory use the same hemolysis scale for all analytes measured on your primary chemistry analyzer?</td>
<td>777</td>
<td>632 (81)</td>
<td>105 (14)</td>
<td>40 (5)</td>
</tr>
<tr>
<td>Does your laboratory use the same hemolysis scale for all analytes measured on your primary chemistry and immunoassay analyzers?</td>
<td>723</td>
<td>503 (70)</td>
<td>165 (23)</td>
<td>55 (8)</td>
</tr>
<tr>
<td>Does your laboratory use the same hemolysis scale for all analytes measured on your primary chemistry analyzer and your backup chemistry analyzer?</td>
<td>672</td>
<td>495 (74)</td>
<td>130 (19)</td>
<td>47 (7)</td>
</tr>
<tr>
<td>Does your laboratory systematically and regularly monitor the percentage of hemolyzed specimens as part of your laboratory's performance improvement plan?</td>
<td>692</td>
<td>325 (47)</td>
<td>321 (46)</td>
<td>46 (7)</td>
</tr>
</tbody>
</table>

Figure 1. Percentage of hemolyzed specimens received by clinical laboratories (N = 772 laboratories).
on their primary and secondary chemistry analyzers, and 503 of 723 (70%) used the same hemolysis scales on the primary chemistry analyzer and immunoassay analyzers. In a few laboratories (325 of 692; 47%), hemolysis was systematically and regularly monitored as part of the laboratory’s performance improvement plan.

In most laboratories (630 of 710; 88%), specimens were rejected depending on the level of hemolysis. However in 50 clinical laboratories (7.0%), all hemolyzed specimens were rejected, and in 25 clinical laboratories (4%), no hemolyzed specimens were rejected.

Sixty-nine specific terms were used to describe hemolysis: 21 terms were used commonly with each term used in at least 10 participating laboratories to classify hemolysis. Slight, moderate, gross, and marked were used in 389, 389, 326, and 161 of 707 laboratories (55%, 55%, 46%, and 23%), respectively (Figure 2). Of the responders, 127 (18%) indicated they used all 3 modifiers, slight, moderate, and gross in their descriptive scales. The most commonly used numeric terms were 1+, 2+, 3+, and 4+ used in 195, 188, 187, and 170 laboratories (28%, 27%, 26%, and 24%), respectively. In the remaining 56 laboratories (8%), 48 additional words, numbers, or phrases described hemolysis.

Figure 3 provides a list and the percentages for 20 cutoff values used to describe specimens rejected for hemolysis in 597 laboratories. In those laboratories, 215 (36%) used a numeric level, and 334 (56%) used a descriptive word to describe the cutoff for specimen rejection. The most common cutoffs that resulted in rejection were moderate hemolysis, used in 182 laboratories (30%), followed by gross hemolysis, which was used in 66 laboratories (11%). The category other was used in 43 additional laboratories (7.2%), where a unique term or additional modifier(s) was added to one of the 20 other terms described in Figure 3. No laboratories used 6+ for rejection, although one laboratory used “greater than 6.”

Figure 4 summarizes practices to detect hemolysis in whole blood specimens in 91 clinical laboratories that use whole blood analyzers not at the point-of-care. Approximately one-half of those using whole blood analyzers measured glucose, potassium, sodium, and chloride, whereas about one-third of those with whole blood systems measured calcium as well as other analytes. Of the 86 laboratories that used whole blood electrolyte analyzers, 48 (56%) did not determine whether specimens were hemolyzed. The most common method, used by 18 (21%) of these
respondents to determine hemolysis, was to centrifuge all whole blood specimens after measurement and then visually determine whether hemolysis was present. In a few clinical laboratories (9 of 86; 10%), personnel stored and then visually inspected all specimens within an hour after measurement to determine whether hemolysis was present. In 5 (6%) of the clinical laboratories, only when potassium was elevated were specimens either centrifuged (3 or 3%) or stored for up to an hour and then visually inspected for hemolysis (2 or 2%). When hemolysis was detected in any of these circumstances, then a comment was added to the quantitative result indicating the specimen was hemolyzed.

**COMMENT**

The overall hemolysis rate in 772 laboratories varied greatly, ranging from less than 1% in 36% of laboratories (n = 276) to 15% or more in 1% of laboratories (n = 8). In more than 70% of laboratories (n = 543), the hemolysis rate was less than 3%, and 9% (n = 71) were unsure of their overall hemolysis rates. This contrasts with a 1997 Q-Probes report in which 0.2% of more than 10 million specimens in 453 clinical laboratories were rejected for hemolysis. A number of participants commented that the hemolysis rates were highest in specimens from their emergency departments; similar findings were described in a number of studies reported in the literature, with hemolysis rates of 3.4%, 12.8%, 12.9%, and 19.8%. It is not surprising to us that in 9% of clinical laboratories (71 of 772), personnel did not know what their overall hemolysis rates were, as common practice is to only monitor and reduce the rates at specific patient locations such as emergency departments where rates are highest.

Some laboratories rejected all hemolyzed specimens. This policy potentially places certain patients at risk such as the case reported by Ismail and colleagues, who described a patient with renal failure and unrecognized intravascular hemolysis who died from the presumed arrhythmia of hyperkalemia because their clinical laboratory had a policy of not measuring potassium values when specimens were hemolyzed. Although the World Health Organization currently recommends that potassium results not be reported when specimens are hemolyzed, we strongly suggest that the laboratory community revisit this issue with consideration of reporting results qualified by noting the presence and degree of hemolysis and an assessment of the effect it may have on the test result, allowing clinicians responsible for the patient’s care to integrate this information into the differential diagnosis and subsequent treatment or to repeat the analysis on another specimen obtained by techniques minimizing in vitro hemolysis.

Most laboratories still used visual scales to grade hemolysis, despite most chemistry analyzers used in central hospital laboratories now measuring hemolysis. Visual estimations of hemolysis are unreliable, and automated methods are preferable. In addition, charts that are used for visual estimation of hemolysis fade, leading to erroneous interpretations. In most laboratories, the description of hemolysis cannot be sent automatically to the patient’s medical record, thereby underestimating the frequency of reporting because technologists working in fast-paced chemistry laboratories could easily forget to perform nonautomated tasks, such as adding a modifier to a patient’s medical record result. Because these hemolysis results cannot be sent automatically to the laboratory information system, these laboratories are probably prevented from using an automated verification system. There is a second consideration regarding nonautomated assessment of hemolysis. Although personnel performing laboratory tests in the United States are required to be checked for color blindness, only 120 of 678 laboratories (18%) evaluated clinical laboratory employees for competency in visually evaluating hemolysis.

Within laboratories, practices for identifying and reporting hemolysis also appeared to vary greatly. Fourteen percent of laboratories (105 of 777) did not use the same hemolysis scale on their primary and secondary chemistry analyzer, and in 23% of clinical laboratories (165 of 723), different hemolysis scales were used on the primary chemistry analyzer and the primary immunoassay analyzer. Different scales emanating from the same laboratory are bound to confuse clinicians. Other examples of variability in hemolysis procedures within the same laboratory included specific reporting procedures that changed according to the analyte measured.

Explanations of hemolysis in some textbooks suggest that quantitative measurements be used to describe hemolysis, and in the United States, these results frequently are reported in milligrams per decaliter. However, in the classic manuscript, Glick et al used 6 numeric, semiquantitative categories, each followed by a plus sign: 0, contains no or trace interferent and 1\(^{\text{st}}\), 2\(^{\text{nd}}\), 3\(^{\text{rd}}\), 4\(^{\text{th}}\), or 5\(^{\text{th}}\). Other workers used descriptive terms that approximately correspond with 7 reportable, numeric categories, such as none (0), none to slight (1), slight (2), slight to moderate (3), moderate (4), moderate to gross (5), and gross (6). Some publications relate descriptive terms to quantitative measurements, such as the use of a scale such as mild (1 g/L), moderate (2.5 g/L), and severe (5 g/L) by Hawkins or the use of slight (50 mg/dL), moderate (100 mg/dL), and gross (200 mg/dL) by Spencer and Roger. Our sampling confirmed the varied classification with 69 different terms, numbers, or combinations of numbers and terms used. Some newer major chemistry analyzers allow users to decide to implement a feature of a quantitative, continuous scale throughout the measurement range to describe hemolysis.

In our experience, we have found that laboratory technicians try to simplify nonautomated processes by having the same rule for all measurements performed on the same specimen type. Hence, it was not surprising to us that most laboratories...
hypokalemic. The Hawkins17 data provide support in critical
to hypokalemic or from hyperkalemic to normokalemia or
compared with serum and that 42% of potassium results
in clinical laboratory and found that hemolysis was more than
5 times higher in whole blood used for blood gases
and that 42% of potassium results
these specimens were downgraded from normokalemic to
hypokalemic or from hyperkalemic to normokalemia or
hypokalemic. The Hawkins15 data provide support in critical
whole blood potassium measurements for the need to
determine whether hemolysis is present.

There is no quick and reliable way to determine whether
hemolysis is present in whole blood specimens. In most
laboratories (56%; 48 of 86) in our study using whole blood
analyzers, laboratory personnel did not take additional steps
to detect whether whole blood specimens were hemolyzed.
Of the few who evaluated hemolysis in whole blood, the
most common practice among 21% of responders was to
centrifuge all whole blood specimens and then visually
examine whether the supernatant was red. The second most
common practice (10%) was to store the whole blood
specimen for up to an hour allowing the red blood cells to
settle and then visually noting whether hemolysis was
present. When potassium was elevated, some laboratories
identified hemolysis in whole blood specimens by storing
the whole blood specimen upright for up to an hour and
then visually examined the specimens for hemolysis (3%), or
inspecting other specimens obtained at the same time for
hemolysis (3%).

One obvious disadvantage of identifying hemolysis in
whole blood specimens is the time required. Visual
inspection required up to an hour before red blood cells
fully settled or required 10 minutes when separation was
hastened by centrifugation. Waiting for these procedures
to occur before visual inspection defeats the main objective of
rapid whole blood testing result reporting.

A second issue with identifying hemolysis in whole blood
specimens is that visual estimation of the amount of
hemolysis has been unreliable because experienced techni-
cologists cannot accurately distinguish between various
concentrations of hemolysis.6,12,13 A third issue is whether or
not hemolysis affects whole blood assays other than
potassium results. Whole blood measurements that are
commonly performed include sodium, calcium, magnesium,
and bilirubin. Effect on these measurements in hemolyzed
whole blood would not always be detected if clinical
laboratory procedures require evaluation of hemolysis only
when potassium was elevated. A potential solution alerting
physicians to hemolysis in a timely manner is to automa-
tically quantitate hemolysis or free hemoglobin in a whole
blood specimen at, or before the time of, measurement of
the whole blood specimen. At this time, there are no US
Food and Drug Administration–approved methods or
instruments available that can make such a measurement.

Widespread use of multianalyte, whole blood point-of
care devices, such as the Abbott iSTAT (Abbott Laborato-
ries, Abbott Park, Illinois) and the Alere epoc (Alere Inc,
Waltham, Massachusetts) blood gas analyzers, as well as
single-test whole blood devices, including glucose meters,
are subject to hemolysis interferences, but based on their
use at locations where no centrifuges are located, proce-
dures used in clinical laboratories to identify hemolysis are
not applicable. The only useful procedure is that if a result is
unexpected and it could have been influenced by hemolysis,
a serum specimen should be sent to the clinical laboratory
for remeasurement.

Quantitative hemoglobin measurements are some of the
most common and accurate measurements made in hospital
clinical laboratories because hemoglobin routinely is mea-
sured by arterial blood gas instruments, complete blood cell
count analyzers, co-oximeters, pulse oximeters, and many
point-of-care devices. There is even a fourth edition of
reference methods for automated hemoglobin measurements
in human blood.18 Despite these resources, laboratorians
continue to use inaccurate visual methods to estimate
hemolysis and report hemolysis using poorly reproducible
terms that confuse clinicians. Finally, most laboratories
continue to accept or reject specimens for measurement of
various analytes using unscientific principles.

We certainly agree with van Rheenen and de Moor19 that
one place where visual estimates of hemoglobin may be the
best that can be achieved is in resource-poor countries
where no automated analyzers are available. In developed
countries, where clinical laboratory instruments that quan-
tify hemoglobin accurately are in the same clinical
laboratory, it is time for laboratorians to standardize policies
and procedures for measurement and reporting of hemo-
lysis. These policies and procedures should include quan-
tification of hemoglobin on a method-by-method basis.

Until standard assessment and reporting occur, variability
among results from identical hemolyzed patient specimens
measured in different clinical laboratories will continue.
Besides standard reporting and consistent assessment of
interferences, we also recommend that instrument
manufacturers consider developing whole blood analyzers
that simultaneously measure hemolysis.

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